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# Response of soil organic carbon and its fractions to natural vegetation restoration in a tropical karst area, southwest China

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The dynamics of soil organic carbon (SOC) and its fractions are important for evaluating the vegetation restoration effect and carbon cycle of the ecosystem. Here, SOC fractions, including light fraction organic carbon (LFOC), heavy fraction organic carbon (HFOC), and labile organic carbon (LOC) fractions (including water-soluble organic carbon, WSOC, readily oxidizable organic carbon, ROC, particulate organic carbon, POC, and microbial biomass carbon, MBC), were investigated at four soil depths under five restoration stages in a tropical karst area in southwest China. This study showed that the content of SOC and its fractions significantly increased with vegetation restoration and decreased with increasing soil depth at each restoration stage ( $p < 0.05$ ). Additionally, LFOC was more sensitive to vegetation restoration, whereas HFOC was the main storage form of SOC. The LOC fractions in the surface soil layer were significantly higher than those in the lower, and the percentages of some LOC fractions (POC/SOC and MBC/SOC) significantly decreased with increasing soil depth, indicating that SOC was more stable in the lower layer than in the surface layer. Correlation analysis showed that SOC was significantly and positively correlated with its fractions. Moreover, SOC and its fraction were positively correlated with soil chemical factors (TN, TP, AP, AK, ECa, EMg,  $\text{NH}_4^+\text{-N}$ , and  $\text{NO}_3^-\text{-N}$ ) and negatively correlated with bulk density (BD) at a significant level ( $p < 0.05$ ). Moreover, redundancy analysis showed that the 12 soil physicochemical factors explained 70.99% of the variation in SOC and its fractions, with AK,  $\text{NH}_4^+\text{-N}$ , and BD being the main factors, explaining 19.38, 17.24, and 10.52% of the variation, respectively. The structural equation mode analysis showed that soil properties, above-ground biomass, and litterfall explained most of the variation in SOC (59%), LFOC (79%), HFOC (81%), and LOC (61%). Soil properties and above-ground biomass significantly affected SOC, LFOC, and HFOC content mainly through indirect effects, while the total phosphorus content of the litterfall could directly

and significantly affect SOC, LFOC, and HFOC content.  $\text{NH}_4^+$ -N and AK of soil factors had direct effects on LFOC and LOC accumulation, respectively. This study provides a valuable perspective for estimating carbon sink potential and constructing carbon sink models in karst areas.

#### KEYWORDS

karst, vegetation restoration, soil organic carbon (SOC), carbon fractions, soil depth

## 1. Introduction

Soil organic carbon (SOC), the largest terrestrial organic carbon pool, has a global storage capacity of approximately 2,344 Gt, which is approximately twice that of the atmospheric carbon pool. A crucial source and sink of atmospheric  $\text{CO}_2$ , SOC plays an important role in the global carbon cycle (Stockmann et al., 2013; Scharlemann et al., 2014). Vegetation restoration can significantly improve SOC sequestration (Li et al., 2012), whereas vegetation degradation and irrational land-use practices can reduce soil carbon sequestration and accelerate greenhouse gas emissions (Lu et al., 2014). SOC sequestration capacity depends on the dynamic balance between the input of carbon sources and the mineralization and decomposition of SOC, influenced by a combination of multiple factors, including climate, restoration period, soil physical structure, and nutrients (Lal, 2004; Laganieri et al., 2010). A broader understanding of the changes in SOC pools during vegetation restoration is required to improve soil carbon sinks and develop effective land management policies.

Soil organic carbon fractions obtained using physical, chemical, and biological methods for grouping SOC attributes better reflect SOC dynamics (Six et al., 2002). For example, light fraction organic carbon (LFOC) and heavy fraction organic carbon (HFOC) are the result of SOC density grouping (Cambardella and Elliott, 1993a). LFOC decomposes rapidly and is significantly affected by vegetation type and litterfall composition, whereas HFOC is relatively stable and is the main fraction of SOC, including the main sink for carbon storage in soils. Additionally, SOC can be classified as labile organic carbon (LOC) and stable organic carbon according to its activity level. LOC, which includes water-soluble organic carbon (WSOC), readily oxidizable organic carbon (ROC), particulate organic carbon (POC), and microbial biomass carbon (MBC), accounts for a relatively small proportion of SOC. However, it can be used to indicate the dynamic characteristics of SOC and directly affects the activity of soil microorganisms, resulting in greenhouse gas emissions (Liang et al., 1997). The content and distribution of LOC fractions are affected by vegetation restoration models and restoration periods (von Lütow et al., 2007).

The global karst area is approximately 22 million  $\text{km}^2$ , accounting for 15% of the total land area on Earth (Ford and Williams, 2007). China has the most widely distributed contiguous karst area in the world, accounting for approximately one-third of the country's land area. The shallow and discontinuous soil layers and highly heterogeneous habitats in karst areas result in complex and fragile karst ecosystems (Xu et al., 2013). High-intensity

human disturbances during the past decades have led to serious damage to karst ecosystems, including vegetation degradation, soil erosion, and land productivity decline, which, in turn, have led to rock desertification, restricting the sustainable development of karst areas in southwest China (Jiang et al., 2014). Since the 1970s, the Chinese government has implemented a series of ecological restoration projects in karst areas, such as the Natural Forest Protection Project, Grain to Green Project, and Rocky Desertification Control Project. Thus, the vegetation cover in karst areas of China has increased significantly, with a combined vegetation cover of 61.4% in 2016, an increase of 3.9% from 2011 and 7.9% from 2005 (Jiang et al., 2014; Tong et al., 2020). Natural closure and afforestation in natural vegetation restoration are two important measures for increasing the green cover in rock desertification areas in karst areas, and natural closure can effectively restore degraded karst ecosystems (Hua et al., 2022).

Natural vegetation restoration is more conducive to carbon sequestration than artificial afforestation in the karst areas of southwest China (Pang et al., 2019). The LOC content increased significantly after karst vegetation restoration; the stability of SOC did not increase in the short term after arable land was retired (Zhao Y. et al., 2021). Arable land in the karst valley of southwest China has the highest SOC content, followed by forests and scrubs. In contrast, the grassland SOC content was the lowest, and SOC content, storage, and fractionation showed decreasing trends with increasing soil depth (Luo et al., 2022). Among the four vegetation types (cropland, grassland, shrubland, and secondary forest) in the karst area of southwestern China, LFOC and HFOC accounted for essentially the same proportion of SOC. This indicated that the stability of SOC was not affected by land use, and exchangeable calcium (ECa) was the main factor influencing the variation in LFOC and HFOC content in subtropical karst areas (Wen et al., 2017). These studies mainly focused on subtropical karst regions, but few studies have explored the dynamics of SOC and its fractions in tropical karst regions. Compared with subtropical karst regions, tropical karst regions are richer in vegetation but have higher temperatures and rainfall, resulting in faster SOC decomposition and carbon cycling (Wang et al., 2016). Additionally, tropical karst regions are often subjected to high levels of soil erosion and loss, affecting the accumulation and retention of SOC in the soil (Zhang et al., 2011). Therefore, more studies are needed to understand how vegetation restoration affects the dynamics of SOC and its fractions in tropical karst areas.

Here, the contents of SOC and its fractions (LFOC, HFOC, WSOC, ROC, POC, and MBC) in different vegetation restoration stages in a tropical karst area in southwest China were investigated to address the following objectives: (1) clarify the changes in

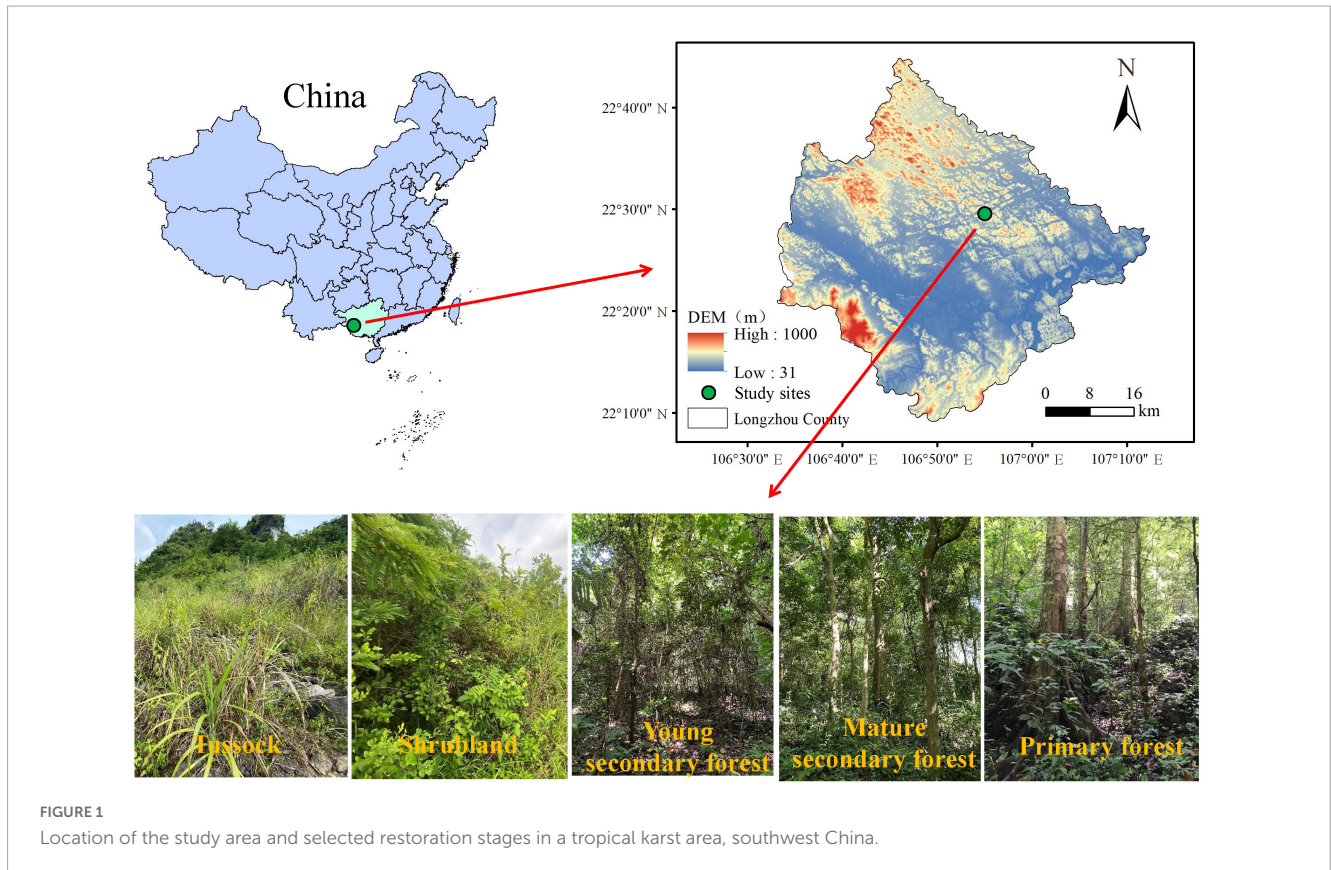


FIGURE 1

Location of the study area and selected restoration stages in a tropical karst area, southwest China.

SOC and its fractions in the soil profile of a tropical karst area with natural vegetation restoration; (2) address the relationships between soil physicochemical factors and SOC and its fractions and determine the main limiting factors for the accumulation of SOC and its fractions; (3) explore the direct and indirect effects of soil, litterfall, and above-ground biomass on the accumulation of SOC and its fractions.

## 2. Materials and methods

### 2.1. Study area

The study area is located in Longzhou County ( $22^{\circ} 8' 49.35''$ – $22^{\circ} 44' 42.30''$ N,  $106^{\circ} 33' 23.33''$ – $107^{\circ} 12' 42.56''$ E), Guangxi Province, southwest China (Figure 1). The area is of tropical monsoon climate with an average annual temperature of  $22^{\circ}\text{C}$ , an absolute maximum temperature of  $39^{\circ}\text{C}$ , and an absolute minimum temperature of  $-3^{\circ}\text{C}$ . The average annual precipitation is 1150–1550 mm and is concentrated from May to October. The geomorphological type is a karst peak-cluster depression landscape with an average elevation of 450 m (asl.) The soil type is alkaline limestone.

### 2.2. Soil sample collection

Five vegetation types, namely tussock (TU,  $2\text{ m} \times 2\text{ m}$ ), shrubland (SL,  $10\text{ m} \times 10\text{ m}$ ), young secondary forest (YSF,

$20\text{ m} \times 20\text{ m}$ ), mature secondary forest (MSY,  $20\text{ m} \times 20\text{ m}$ ), and primary forest (PF,  $20\text{ m} \times 20\text{ m}$ ), were selected as natural successional sequences using a spatial rather than temporal approach in August 2021. The selected natural vegetation grew under similar climatic conditions. A total of 20 plots with four replicate plots in each vegetation restoration stage were set up with the similar geographical conditions; Table 1 shows the basic information of these sampling sites. In each sampling plot, soil samples were collected from four soil depths (i.e., 0–10, 10–20, 20–30, and 30–50 cm) in triplicate, and the three replicates of soil samples from the same soil layer were mixed as one sample. Finally, a total of 80 samples were collected and placed in an insulated bag and brought to the laboratory for storage at  $4^{\circ}\text{C}$ . After removing roots and debris from the soil and passing through a 2 mm soil sieve, each soil sample was divided into two parts: one fresh soil samples was used for the determination of WSOC and MBC, while the other samples were naturally air-dried to measure the SOC, LFOC, HFOC, ROC, and POC contents. Before collecting soil samples, the thickness of the ground litterfall at each sampling plot was measured and the ground litterfall within  $0.5\text{ m} \times 0.5\text{ m}$  square area in the sampling plot was collected for calculating the litterfall yield and determining the content of litterfall organic carbon (Litter C), total nitrogen (Litter N), and total phosphorus (Litter P).

### 2.3. Soil analysis

Soil bulk density (BD) was determined using the cutting ring method in the field (Hossain et al., 2015), soil electrical conductivity

TABLE 1 Basic information of the study area and sampling sites.

Restoration stages	Plots	Latitude	Longitude	Altitude (m)	Slope degree (°)	Slope aspect (°)	Restoration years (a)	Canopy cover (%)	Above-ground biomass ( $10^3 \text{ kg}\cdot\text{hm}^{-2}$ )	Dominant species
Tussock	TU-1	106°56'29.70''	22°27'41.13''	307	2	296	5	28	4.25	<i>Chromolaena odorata</i> , <i>Bidens pilosa</i> , <i>Miscanthus floridulus</i> , <i>Imperata cylindrica</i>
	TU-2	106°56'28.81''	22°27'42.02''	306	5	113	5	28	5.79	
	TU-3	106°56'28.19''	22°27'41.22''	306	5	126	5	30	3.46	
	TU-4	106°56'28.18''	22°27'40.20''	307	3	130	5	33	4.38	
Shrubland	SL-1	106°56'30.86''	22°27'46.45''	305	10	212	15	45	12.38	<i>Rhus chinensis</i> , <i>Desmos chinensis</i> , <i>Alchornea trewioides</i> , <i>Breynia fruticosa</i>
	SL-2	106°56'29.29''	22°27'47.20''	303	14	205	15	43	9.74	
	SL-3	106°56'27.42''	22°27'47.34''	301	10	130	15	45	14.52	
	SL-4	106°56'26.38''	22°27'45.66''	303	12	145	15	48	23.98	
Young secondary forest	YSF-1	106°56'37.89''	22°27'55.12''	317	27	40	30	65	69.94	<i>Horsfieldia hainanensis</i> , <i>Orophea polycarpa</i> , <i>Metadina trichotoma</i> , <i>Cleistanthus sumatranus</i>
	YSF-2	106°56'41.44''	22°27'56.44''	316	26	256	30	63	74.26	
	YSF-3	106°56'38.83''	22°27'58.14''	322	27	215	30	65	94.04	
	YSF-4	106°56'36.03''	22°27'58.44''	323	29	110	30	68	65.01	
Mature secondary forest	MSF-1	106°56'42.95''	22°28'1.37''	324	18	90	60	70	85.22	<i>Streblus tonkinensis</i> , <i>Cleistanthus sumatranus</i> , <i>Deutzianthus tonkinensis</i> , <i>Vitex kwangsiensis</i>
	MSF-2	106°56'46.93''	22°28'1.01''	313	23	286	60	75	147.46	
	MSF-3	106°56'46.93''	22°28'4.30''	317	20	253	60	73	84.08	
	MSF-4	106°56'42.17''	22°28'4.90''	334	23	136	60	73	127.18	
Primary forest	PF-1	106°56'28.97''	22°28'3.05''	334	13	270	> 100	78	152.92	<i>Excentrodendron tonkinense</i> , <i>Cephalomappa sinensis</i> , <i>Diplodiscus trichospermus</i> , <i>Cleistanthus sumatranus</i>
	PF-2	106°56'30.63''	22°28'5.19''	342	18	265	> 100	78	167.25	
	PF-3	106°56'28.89''	22°28'9.22''	361	15	213	> 100	75	128.54	
	PF-4	106°56'26.49''	22°28'5.76''	345	10	98	> 100	73	182.95	

(EC) using the electrode method (Rhoades and Corwin, 1981), and soil pH using the potentiometric method (Bao, 2013). Soil effective phosphorus (AP) was determined using sodium bicarbonate leaching and molybdenum antimony anti-spectrophotometric method and soil effective potassium (AK) content using flame atomic absorption spectrophotometry. Total nitrogen (TN) and Litter N were determined using the Kjeldahl method, total phosphorus (TP) and Litter P using alkali fusion-molybdenum antimony anti-spectrophotometry, and total potassium (TK) using atomic absorption spectrophotometry. ECa was determined using ammonium acetate exchange-EDTA complex titration and exchangeable magnesium (EMg) using ammonium acetate exchange-atomic absorption spectrophotometry. Ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) and nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ) were determined using nano-reagent and UV spectrophotometry, respectively. The above methods were used to determine the soil physicochemical properties, as described by Bao (2013). The mean values of the above 12 soil properties are shown in **Supplementary Table 1**, and the properties of litterfall in the sample plots are shown in **Supplementary Table 2**.

The SOC and Litter C were determined by oxidation spectrophotometry using potassium dichromate (Bao, 2013). LFOC and HFOC were determined using NaI density separation (Janzen et al., 1992). The separation specific gravity used here was 1.7, with a specific gravity of less than 1.7 for LFOC and greater than 1.7 for HFOC (Janzen et al., 1992). WSOC was extracted using deionized water (water-soil ratio of 2:1) and extracted with a 0.45  $\mu\text{m}$  membrane; the filtrate was determined on a TOC analyzer (Springob and Kirchmann, 2002). ROC was determined using the  $\text{KMnO}_4$  oxidation method (Blair et al., 1995), POC using the sodium hexametaphosphate separation method (Cambardella and Elliott, 1993b), and MBC using the chloroform fumigation extraction method (Wang et al., 2003).

## 2.4. Statistical analysis

Two-way analysis of variance (ANOVA) was used to examine the effects of vegetation restoration and soil depth on the content of SOC and its fractions. Data were tested for chi-square and multiple comparisons between means using the least significant difference method at a significance level of 0.05. Pearson's correlation analysis was used to analyze the correlation between soil physicochemical factors and SOC and its fractions. Redundancy analysis (RDA) was used to explore the main factors affecting SOC and its fractions and calculate the explanation rate of each environmental factor. Variables with a variance inflation factor (VIF) greater than 10 were removed by detecting multicollinearity among environmental factors before performing RDA. Structural equation modeling was used to further reveal the direct and indirect pathways through which regulatory factors influence changes in SOC and its fractions. Statistical data were expressed as mean  $\pm$  standard error (mean  $\pm$  SE). ANOVA was performed using SPSS (version 24.0; SPSS Inc., Chicago, IL, USA). The correlation analysis, RDA analysis, contribution of each explanatory variable, and construction of the structural equation model were based on the psych (Revelle, 2017), vegan (Oksanen et al., 2016), rdacca.hp (Lai et al., 2022), and piecewiseSEM (Lefcheck, 2016) packages in R 4.1.3, respectively.

## 3. Results

### 3.1. Changes in SOC content at different restoration stages

As shown in **Figure 2A**, **Table 2**, vegetation restoration and soil depth had significant effects on SOC content. During vegetation restoration, the SOC content of the 0–50 cm soil layer varied from 13.00 to 46.19  $\text{g}\cdot\text{kg}^{-1}$ . Vegetation restoration only had a significant effect on the SOC of the 0–10 cm soil layer, showing that MSF and PF were significantly higher than TU and SL, YSF was significantly higher than TU ( $p < 0.05$ ), and the SOC content tended to increase with vegetation restoration. In the soil profile, the highest SOC content was in the 0–10 cm soil layer at each restoration stage, and SOC content gradually decreased with increasing soil depth.

### 3.2. Changes in LFOC and HFOC contents at different restoration stages

The LFOC and HFOC contents were significantly affected by vegetation restoration and different soil depths; LFOC and HFOC contents varied from 1.31–8.57 to 11.69–38.07  $\text{g}\cdot\text{kg}^{-1}$ , respectively (**Figures 2B, C** and **Table 2**). The LFOC content was highest in all soil layers at the PF stage, and vegetation restoration only had a significant effect on the HFOC content in the 0–10 cm soil layer, indicating that MSF was significantly higher than TU ( $p < 0.05$ ); the LFOC and HFOC contents tended to increase with vegetation restoration. In the soil profile, LFOC and HFOC contents in the 0–10 cm soil layer were significantly higher than those in the other soil layers ( $p < 0.05$ ), and the LFOC and HFOC contents gradually decreased with increasing soil depth.

### 3.3. Changes in LOC content at different restoration stages

Vegetation restoration and soil depth significantly affected each LOC fraction (WSOC, **Figure 2D**; ROC, **Figure 2E**; POC,

TABLE 2 Two-way ANOVA of single and interactions effects of soil depth and recovery stage on soil organic carbon (SOC) and its fractions.

Factors	Recovery stage	Soil depth	Recovery stage x Soil depth
SOC	5.75**	38.41***	0.34
LFOC	20.57***	74.35***	1.69
HFOC	4.26**	33.71***	0.25
WSOC	8.89***	38.62***	0.98
ROC	3.58*	29.54***	0.58
POC	8.62***	46.99***	1.15
MBC	22.81***	102.09***	4.11***

SOC, soil organic carbon; LFOC, light fraction organic carbon; HFOC, heavy fraction organic carbon; WSOC, water-soluble organic carbon; ROC, readily oxidizable organic carbon; POC, particulate organic carbon; MBC, microbial biomass carbon. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

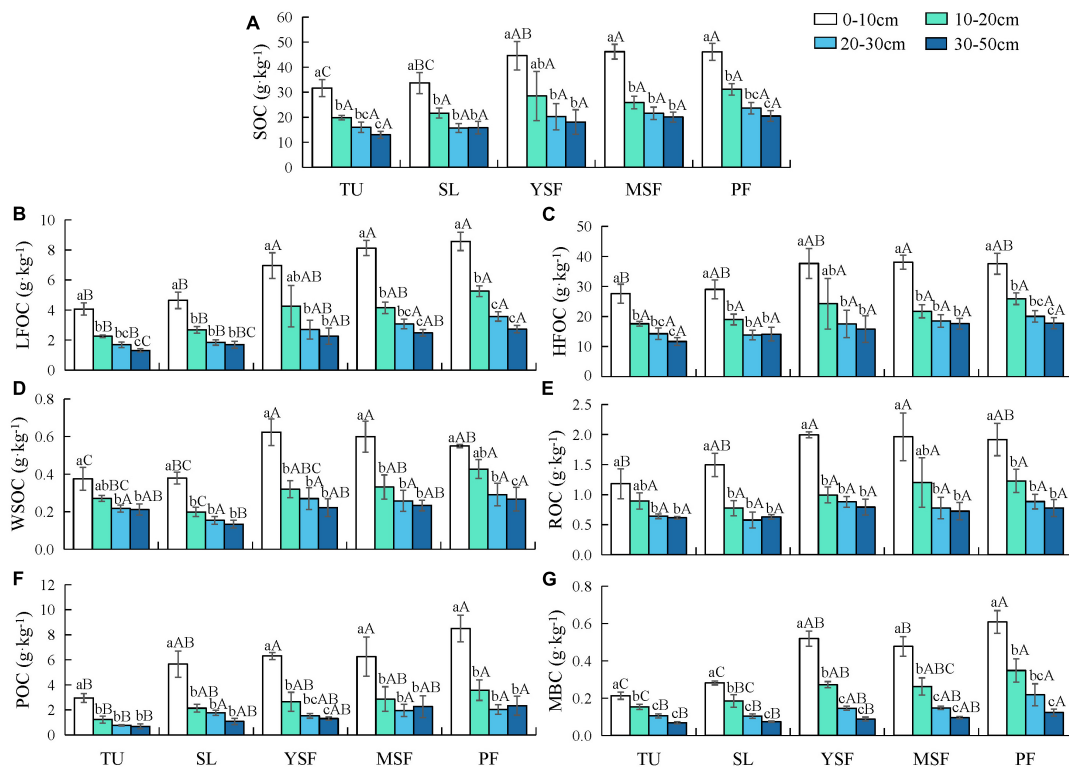


FIGURE 2

Distribution of (A) soil organic carbon (SOC), (B) light fraction organic carbon (LFOC), (C) heavy fraction organic carbon (HFOC), (D) water-soluble organic carbon (WSOC), (E) readily oxidizable organic carbon (ROC), (F) particulate organic carbon (POC), and (G) microbial biomass carbon (MBC) content in different vegetation restoration stages. TU, tussock, SL, shrubland, YSF, young secondary forest, MSF, mature secondary forest, PF, primary forest. Lowercase letters indicated significant differences amongst the same vegetation restoration stages of different soil layers, and capital letters indicated significant differences amongst different vegetation restoration stages in the same soil layers at the 0.05 level. Mean value  $\pm$  standard error.

Figure 2F; MBC, Figure 2G). WSOC content ranged from 0.13 to 0.62 g·kg<sup>-1</sup> at each restoration stage, with the highest content at the YSF stage in the 0–10 cm soil layer and at the PF stage in all 10–50 cm soil layers. ROC content varied from 0.62 to 1.99 g·kg<sup>-1</sup> and was highest in the YSF stage in the 0–10 and 30–50 cm soil layers, and the WSOC content was highest in the PF stage in both the 10–30 cm soil layers. POC content ranged from 0.69 to 8.49 g·kg<sup>-1</sup> during vegetation restoration, and the MBC content varied from 0.07 to 0.61 g·kg<sup>-1</sup> during vegetation restoration, with the highest POC and MBC contents in all soil layers at the PF stage. Overall, each LOC fraction increased with vegetation recovery.

In the soil profile, the highest content of each LOC fraction was in the 0–10 cm soil layer, and the content of each LOC fraction decreased with increasing soil depth, as follows: the contents of WSOC, ROC, POC, and MBC in the TU, YSF, MSF, and PF stages 0–10 cm were significantly higher than those in the 20–30 and 30–50 cm soil layers ( $p < 0.05$ ); the WSOC, ROC, POC, and MBC contents in the 0–10 cm layer in SL stage were significantly higher than those in the other three soil layers ( $p < 0.05$ ).

### 3.4. Changes in proportion of SOC fractions at different restoration stages

As shown in Table 3, the variation in LFOC/SOC at each restoration stage ranged from 10.14 to 18.59%, and HFOC/SOC

fluctuated from 81.41 to 89.86%. The LFOC/SOC and HFOC/SOC increased and decreased, respectively, with vegetation restoration. In the soil profile, LFOC/SOC decreased with the deepening of the soil layer, whereas the distribution pattern of HFOC/SOC was the opposite to that of LFOC/SOC. The variation in WSOC/SOC at each restoration stage ranged from 0.86 to 1.66%, and ROC/SOC fluctuated from 3.58 to 5.41%, both of which showed no obvious variation pattern with soil depth. The variation in POC/SOC ranged from 5.15 to 16.76% and MBC/SOC fluctuated from 0.49 to 1.31% at each restoration stage, showing a decreasing trend with increasing soil depth. In general, WSOC/SOC and ROC/SOC did not significantly change with vegetation restoration ( $p > 0.05$ ), whereas POC/SOC and MBC/SOC increased ( $p < 0.05$ ).

### 3.5. Relationships between SOC and its fractions and soil physicochemical properties

As shown in Figure 3, the SOC and its fractions were significantly positively correlated. A significant positive correlation was observed between soil physicochemical properties (EC, AK, NO<sub>3</sub><sup>-</sup>-N, TN, NH<sub>4</sub><sup>+</sup>-N, AP, EMg, ECa, TP, and TK) and SOC, LFOC, HFOC, POC, and MBC. In contrast, SOC, LFOC, HFOC, POC, and MBC were highly negatively correlated with BD. WSOC

and ROC were significantly positively correlated with EC, AK,  $\text{NO}_3^-$ -N, TN,  $\text{NH}_4^+$ -N, AP, EMg, ECa, TP, TK, and pH and were significantly negatively correlated with BD.

The RDA results in **Figure 4** show that RDA1 and RDA2 explained 92.26 and 5.56% of the variance, respectively. All the soil factors explained 70.99% of the variance in SOC and its fractions (**Table 4**), with AK,  $\text{NH}_4^+$ -N, and BD being the main influencing factors, explaining 19.38, 17.24, and 10.52% of the variance, respectively.

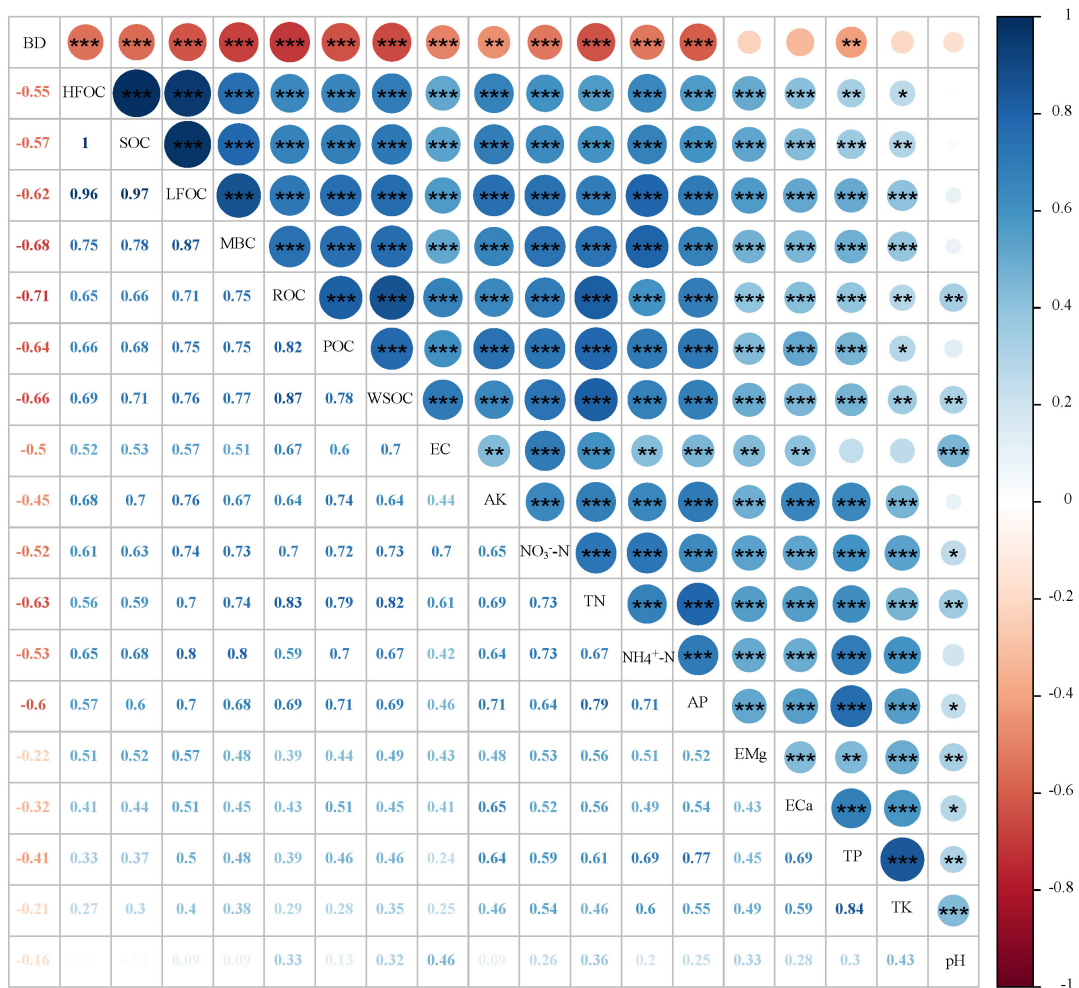
### 3.6. Driving mechanisms of changes in SOC and its fractions

PiecewiseSEM was used to reveal the direct and indirect pathways through which regulatory factors influence changes in SOC and its fractions (**Figure 5**). Soil properties, above-ground biomass, and litterfall explained most of the variation in SOC (59%), LFOC (79%), HFOC (81%), and LOC (61%). Soil properties and above-ground biomass significantly affected SOC, LFOC, and

**TABLE 3** Percentage of different fractions of soil organic carbon and results of two-way ANOVA of single and interactions effects of soil depth and recovery stage on percentage of different fractions of soil organic carbon.

Restoration stages	Soil depth (cm)	LFOC/SOC (%)	HFOC/SOC (%)	WSOC/SOC (%)	ROC/SOC (%)	POC/SOC (%)	MBC/SOC (%)
Tussock	0~10	12.88 ± 0.10 aE	87.12 ± 0.10 dA	1.18 ± 0.12 bA	3.68 ± 0.54 aA	9.53 ± 1.28 aB	0.70 ± 0.10 aC
	10~20	11.36 ± 0.06 bE	88.64 ± 0.06 cA	1.36 ± 0.05 abA	4.57 ± 0.76 aA	6.39 ± 1.66 aA	0.77 ± 0.04 aA
	20~30	10.67 ± 0.21 cE	89.33 ± 0.21 bA	1.39 ± 0.11 abA	4.13 ± 0.33 aA	5.15 ± 0.83 aB	0.67 ± 0.06 aA
	30~50	10.14 ± 0.21 dC	89.86 ± 0.21 aA	1.66 ± 0.23 aA	4.86 ± 0.44 aA	5.74 ± 1.85 aA	0.55 ± 0.10 aA
	Average	11.26 ± 0.28 C	88.74 ± 0.28 A	1.40 ± 0.08 A	4.31 ± 0.27 A	6.70 ± 0.78 B	0.67 ± 0.04 B
Shrubland	0~10	13.85 ± 0.13 aD	86.15 ± 0.13 cB	1.15 ± 0.08 aA	4.43 ± 0.09 aA	16.76 ± 2.46 aAB	0.90 ± 0.17 aBC
	10~20	12.38 ± 0.13 bD	87.62 ± 0.13 bB	0.91 ± 0.05 bA	3.52 ± 0.45 aA	10.55 ± 2.56 abA	0.91 ± 0.23 aA
	20~30	11.78 ± 0.18 bD	88.22 ± 0.18 bB	0.98 ± 0.06 abA	3.58 ± 0.61 aA	11.71 ± 2.09 abA	0.68 ± 0.08 aA
	30~50	10.85 ± 0.32 cC	89.15 ± 0.32 aA	0.86 ± 0.08 bA	4.21 ± 0.49 aA	8.22 ± 3.26 bA	0.51 ± 0.09 aA
	Average	12.21 ± 0.30 C	87.79 ± 0.30 A	0.97 ± 0.04 B	3.93 ± 0.23 A	11.81 ± 1.42 A	0.75 ± 0.08 AB
Young secondary forest	0~10	15.63 ± 0.08 aC	84.37 ± 0.08 bC	1.47 ± 0.29 aA	4.72 ± 0.66 aA	14.83 ± 2.06 aAB	1.22 ± 0.15 aAB
	10~20	15.21 ± 0.26 aC	84.79 ± 0.26 bC	1.42 ± 0.33 aA	4.61 ± 1.27 aA	13.41 ± 5.69 aA	1.27 ± 0.32 aA
	20~30	13.53 ± 0.26 bC	86.47 ± 0.26 aC	1.63 ± 0.51 aA	5.22 ± 1.21 aA	8.68 ± 1.95 aAB	0.82 ± 0.13 abA
	30~50	12.77 ± 0.36 bAB	87.23 ± 0.36 aAB	1.56 ± 0.49 aA	5.41 ± 1.43 aA	8.64 ± 1.99 aA	0.58 ± 0.15 bA
	Average	14.29 ± 0.32 B	85.71 ± 0.32 B	1.52 ± 0.19 A	4.99 ± 0.53 A	11.39 ± 1.65 A	0.97 ± 0.12 A
Mature secondary forest	0~10	17.59 ± 0.04 aB	82.41 ± 0.04 dD	1.31 ± 0.19 aA	4.35 ± 0.96 aA	13.52 ± 3.18 aAB	1.05 ± 0.15 aABC
	10~20	16.08 ± 0.13 bB	83.92 ± 0.13 cD	1.42 ± 0.47 aA	5.41 ± 2.61 aA	12.92 ± 6.27 aA	1.03 ± 0.15 aA
	20~30	14.29 ± 0.13 cB	85.71 ± 0.13 bD	1.27 ± 0.35 aA	3.88 ± 1.15 aA	9.12 ± 2.35 aAB	0.72 ± 0.11 abA
	30~50	12.38 ± 0.15 dB	87.62 ± 0.15 aB	1.24 ± 0.29 aA	3.96 ± 1.27 aA	12.69 ± 6.28 aA	0.49 ± 0.05 bA
	Average	15.09 ± 0.51 AB	84.91 ± 0.51 BC	1.31 ± 0.15 AB	4.40 ± 0.75 A	12.06 ± 2.22 A	0.82 ± 0.08 AB
Primary forest	0~10	18.59 ± 0.04 aA	81.41 ± 0.04 dE	1.21 ± 0.09 aA	4.13 ± 0.47 aA	18.64 ± 2.64 aA	1.31 ± 0.05 aA
	10~20	16.88 ± 0.06 bA	83.12 ± 0.06 cE	1.38 ± 0.15 aA	3.87 ± 0.33 aA	11.56 ± 2.91 abA	1.09 ± 0.11 abA
	20~30	15.17 ± 0.11 cA	84.83 ± 0.11 bE	1.20 ± 0.17 aA	3.74 ± 0.40 aA	8.67 ± 1.64 bAB	0.88 ± 0.15 bcA
	30~50	13.36 ± 0.14 dA	86.64 ± 0.14 aC	1.25 ± 0.21 aA	3.71 ± 0.39 aA	10.82 ± 3.26 abA	0.63 ± 0.14 cA
	Average	16.00 ± 0.50 A	84.00 ± 0.50 C	1.26 ± 0.07 AB	3.86 ± 0.18 A	12.42 ± 1.54 A	0.98 ± 0.08 A
Average		13.77 ± 0.26	86.23 ± 0.26	1.29 ± 0.06	4.30 ± 0.20	10.88 ± 0.73	0.84 ± 0.04
Two-way ANOVA		-	-	-	-	-	-
Recovery stage		487.836***	487.836***	2.404	0.86	2.195	3.629**
Soil depth		419.607***	419.607***	0.029	0.114	3.587*	13.08***
Recovery stage × Soil depth		11.425***	11.425***	0.271	0.278	0.324	0.564

Lowercase letters indicated significant differences amongst the same vegetation restoration stages of different soil layers, and capital letters indicated significant differences amongst different vegetation restoration stages in the same soil layers at the 0.05 level. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .



**FIGURE 3** Correlation analysis between soil organic carbon (SOC) and its fractions and soil physicochemical properties. BD, bulk density; HFOC, heavy fraction organic carbon; SOC, soil organic carbon; LFOC, light fraction organic carbon; MBC, microbial biomass carbon; ROC, readily oxidizable organic carbon; POC, particulate organic carbon; WSOC, water-soluble organic carbon; EC, electrical conductivity; AK, available potassium; NO<sub>3</sub><sup>-</sup>-N, nitrate nitrogen; TN, total nitrogen; NH<sub>4</sub><sup>+</sup>-N, ammonium nitrogen; AP, available phosphorus; EMg, exchangeable magnesium; Eca, exchange of calcium; TP, total phosphorus; TK, total potassium. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

HFOC content mainly through indirect effects, while Litter P content could directly and significantly affect SOC, LFOC, and HFOC content (*p* < 0.05) (Figures 5A–C). Additionally, NH<sub>4</sub><sup>+</sup>-N and AK of soil factors directly affected the LFOC and LOC accumulation, respectively (Figures 5B, D).

## 4. Discussion

### 4.1. Effect of vegetation restoration on SOC

Soil organic carbon content is determined by the dynamic balance between the input of carbon sources from plant residues, root secretions, and root biomass and the loss of carbon due to soil respiration and erosive transport by water. The accumulation process is influenced by a combination of factors such as soil development, vegetation type, and anthropogenic disturbance

(Laganiere et al., 2010). In this study, the variation of SOC content ranged from 13.00 to 46.19 g·kg<sup>-1</sup>, which is similar to the findings of Pang et al. (2019) and Hu and Lan (2020) in the subtropical karst region. Although tropical and subtropical karst regions differ in climate and vegetation, both are characterized by limestone bedrock and distinctive landforms, resulting in a similar range of variation in SOC content. Additionally, in the 0–50 cm soil layer, the average SOC contents of TU, SL, YSF, MSF, and PF stages were 20.11, 21.71, 27.85, 28.44, and 30.35 g·kg<sup>-1</sup>, respectively, and the SL, YSF, MSF, and PF stages increased 0.08, 0.38, 0.41, and 0.51 times, respectively. The largest increase in SOC content was observed when recovering from SL to YSF. YSF is the initial stage of seasonal rainforests with high biomass, resulting in a significant increase in SOC content from SL to YSF. Previous studies have also shown that SOC content increases greatly through vegetation restoration (Yang et al., 2016), such as an increase of 35–100% after reforestation (Johnson, 1992).

We found that the SOC content at each vegetation restoration stage gradually decreased with increasing soil depth. The SOC



content at the 0–10 cm depth was the highest among the four soil depths, which was 39.35% of the 0–50 cm layer. This finding is similar to the results of [Hu and Lan \(2020\)](#) and might be explained by the enriched distribution of litterfall and roots in the surface soil (0–10 cm depth). The decomposition of litterfall and roots results in enriched humus in the surface soil with an evident surface aggregation phenomenon ([Wang et al., 2014](#)). Additionally, high moisture, abundant microorganisms, frequent disturbances, abundant litterfall, and plant roots in the surface soil are helpful for the accumulation of SOC ([Barreto et al., 2011](#)). Lower SOC is mainly influenced by root residues and secretions, with less effect of litterfall and animal activity ([Fontaine et al., 2007](#)), resulting in little change in lower SOC.

## 4.2. Effects of vegetation restoration on LFOC and HFOC fractions

Light fraction organic carbon is an active soil carbon pool with a high turnover rate and rapid mineralization and decomposition. In comparison, as the stable carbon pool of the soil, HFOC is organic carbon combined with soil mineral colloids, which is not sensitive to external disturbances ([von Lützwow et al., 2007](#)). In this study, vegetation restoration increased the soil LFOC and HFOC contents. In the 0–50 cm soil layer, the average LFOC contents in TU, SL, YSF, MSF, and PF stages were 2.33, 2.71, 4.04, 4.06, and 5.03 g·kg<sup>-1</sup>, respectively. From TU to SL, YSF, MSF, and PF, the LFOC content increased 0.16, 0.74, 0.91, and 1.16 times, respectively. Compared with SOC, LFOC has a faster accumulation rate and is more sensitive than SOC in response to vegetation restoration ([John et al., 2005](#)). From TU to SL, YSF, MSF, and PF, the average content of HFOC was 17.78, 19.00, 23.81, 23.98, and 25.32 g·kg<sup>-1</sup>, respectively. Compared with the TU stage, the SL, YSF, MSF, and PF stages increased by 0.07, 0.34, 0.35, and 0.42 times, respectively. A similar increase in SOC and an HFOC/SOC value of 86.23% indicated that the soil carbon pool was dominated by the more stable HFOC ([Zhao et al., 2016](#)). LFOC tended to increase more rapidly during the early stages of vegetation restoration, whereas HFOC tended to increase more slowly over time, resulting in an increase in LFOC/SOC with vegetation recovery, whereas HFOC/SOC decreased. This is because labile organic matter is more easily decomposed and contributes more to short-term changes in SOC, whereas the formation and stabilization of humic substances is a slower process ([Boone, 1994](#)).

The LFOC content and LFOC/SOC tended to decrease with the deepening of the soil layer because the upper soil layers received more organic matter inputs from plant residues and other sources, increasing the amount of labile carbon available to microorganisms. Thus, the LFOC content and LFOC/SOC ratio tended to be higher in the surface soil layers. However, as organic matter decomposed and was consumed by microorganisms, the LFOC content and LFOC/SOC ratio tended to decrease with depth. This is because deeper soil layers have less organic matter input and are more affected by physical and chemical processes that can lead to the loss of labile carbon ([Deng et al., 2022](#)). HFOC content decreased with increasing soil depth, which was related to more abundant SOC in the surface soil. Additionally, the high infiltration of surface soil water results in arid surface soil in karst areas, limiting

microbial activity and litterfall decomposition. This leads to the decomposition rate of litterfall being smaller than the input rate of litterfall and accumulating with increasing years of vegetation restoration, resulting in a decrease in HFOC and LFOC content with increasing soil depth ([Neilson et al., 2017](#)). The HFOC/SOC ratio tended to increase with depth because deeper soil layers are more stable and provide a better environment for the formation and accumulation of humic substances. This is because humic substances are more resistant to decomposition and less affected by physical and chemical processes that can lead to the loss of organic carbon ([Li et al., 2022](#)).

## 4.3. Effects of vegetation restoration on LOC fractions

Water-soluble organic carbon is an organic carbon source directly available to soil microorganisms, and its ease of decomposition and mineralization is an important pathway for SOC loss ([Maie et al., 2004](#)). Here, the WSOC content gradually increased with vegetation restoration, probably because the increased vegetation cover reduced the leaching and scouring effects of rainwater. In contrast, the decomposition of plant litterfall and root secretions increased the input of SOC, thereby significantly increasing the WSOC content. The variation of WSOC/SOC in this study ranged from 0.86 to 1.66%, which was lower than the results of [Pang et al. \(2019\)](#). As an important indicator of soil reactive organic carbon (ROC), changes in ROC can better reflect the relationship between soil reactive organic carbon and SOC changes and have a stronger sensitivity to SOC changes ([Liang et al., 1997](#)). We found that vegetation restoration had a significant effect on the ROC content in the 0–10 cm soil layer. Leaching erosion is the determining factor for differences in the ROC content of topsoil ([Chen et al., 2017](#)). The average annual rainfall in the study area was 1,150–1,550 mm, and

TABLE 4 Interpretation rate of a single environmental factor in redundancy analysis.

Soil environment factor	Volume of explanation (%)	Explanation rate (%)	P-value
AK	13.76	19.38	0.001
NH <sub>4</sub> <sup>+</sup> -N	12.24	17.24	0.001
BD	7.47	10.52	0.001
NO <sub>3</sub> <sup>-</sup> -N	6.39	9.00	0.001
EMg	5.85	8.24	0.001
AP	5.81	8.18	0.001
EC	4.98	7.01	0.001
TN	4.76	6.70	0.001
TP	4.47	6.30	0.001
Eca	2.33	3.28	0.001
pH	1.73	2.44	0.001
TK	1.20	1.69	0.006
Total	70.99	100.00	-

AK, available potassium; NH<sub>4</sub><sup>+</sup>-N, ammonium nitrogen; BD, bulk density; NO<sub>3</sub><sup>-</sup>-N, nitrate nitrogen; EMg, exchangeable magnesium; AP, available phosphorus; EC, electrical conductivity; TN, total nitrogen; TP, total phosphorus; Eca, exchange of calcium; TK, total potassium. Mean value ± standard error.

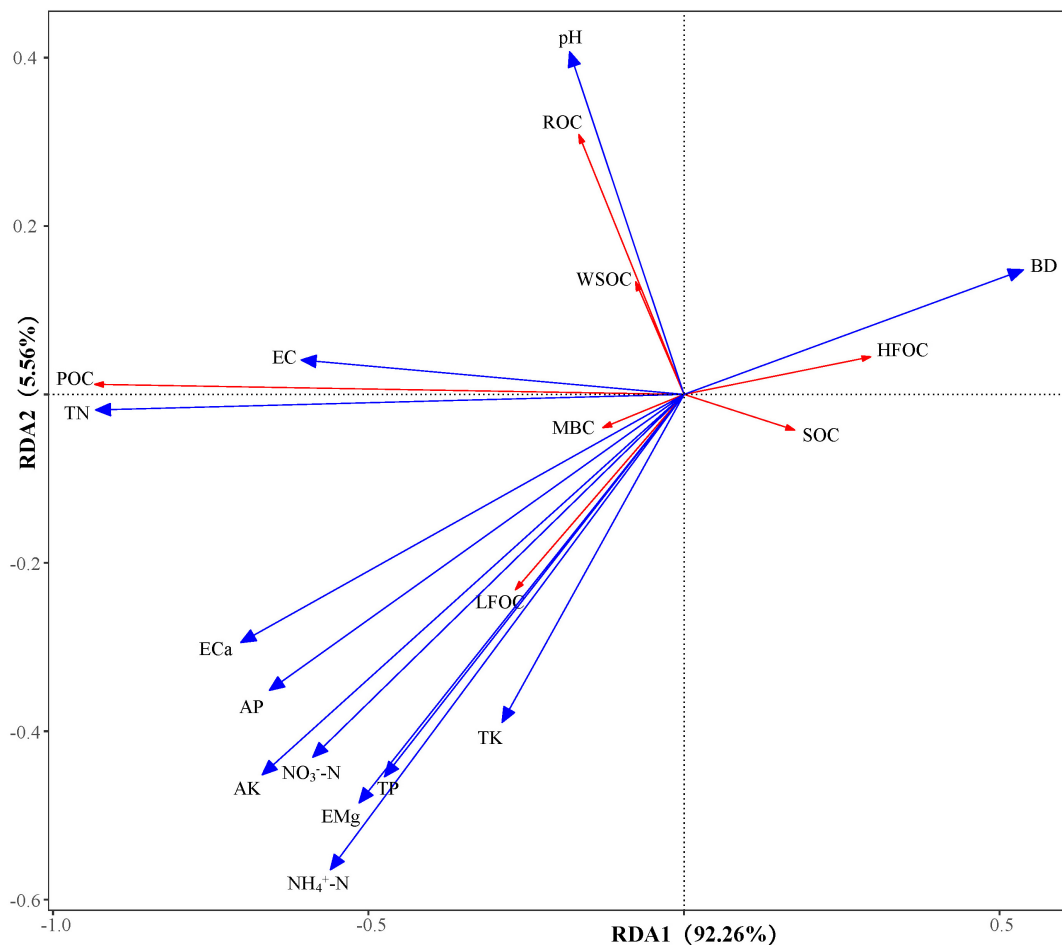


FIGURE 4

Redundancy analysis of SOC and its fractions with soil physicochemical properties. Response variable: SOC, soil organic carbon; LFOC, light fraction organic carbon; HFOC, heavy fraction organic carbon; WSOC, water-soluble organic carbon; ROC, readily oxidizable organic carbon; POC, particulate organic carbon; MBC, microbial biomass carbon. Explanatory variable: BD, bulk density; EC, electrical conductivity; AP, available phosphorus; AK, available potassium; ECa, exchange of calcium; EMg, exchangeable magnesium; NH<sub>4</sub><sup>+</sup>-N, ammonium nitrogen; NO<sub>3</sub><sup>-</sup>-N, nitrate nitrogen; TN, total nitrogen; TP, total phosphorus; TK, total potassium. The red line indicates the response variable, and the blue line indicates the explanatory variable.

the leaching erosion was strong. The restoration of vegetation improved the retention of rainwater by the canopy, litterfall, and roots, gradually decreasing the topsoil leaching erosion. Therefore, the ROC content of the topsoil increased significantly after vegetation restoration. Additionally, the ROC/SOC ratio did not significantly change with vegetation recovery, probably because of the high levels of erosion and soil loss in tropical karst areas (Chen et al., 2017). POC is the SOC bound to soil grit and reflects the amount of non-conserved organic carbon in the soil, representing a certain percentage of the active organic carbon pool (Díaz-Pinés et al., 2011). This study showed that the soil POC content and POC/SOC ratio tended to increase with revegetation. Chan (1997) found that the restoration from cropland to grassland with an increase in plant litterfall led to an increase in the POC content of the soil. Litterfall and plant secretions provide carbon sources for soil microorganisms, and soil microorganisms, as decomposers, provide nutrients for plant growth, which is important for maintaining plant diversity, ecosystem stability, and productivity (Jiang and Song, 2010). In

this study, the MBC content and MBC/SOC tended to increase with vegetation restoration. Hu and Guo (2012) suggested that apoplastic and phyto secretory materials increase with vegetation restoration, while apoplastic materials reduce surface evaporation and increase soil water content, facilitating the accumulation of MBC.

The LOC fraction contents (WSOC, ROC, POC, and MBC) increased during vegetation restoration. At the early stage of vegetation restoration, vegetation composition and diversity are relatively simple, and the organic matter input to the soil from above-ground vegetation is relatively low (O'Brien and Jastrow, 2013). After vegetation restoration, the community structure became diversified and stable, the amount of litterfall, fine roots, and root secretions increased significantly, organic matter returned to the soil, and soil microbial activity increased, leading to a rapid increase in the LOC fraction content with an increase in vegetation restoration (Xue et al., 2009). The LOC fraction contents showed a decreasing trend with increasing soil depth, and the percentages of some active organic carbon fractions (POC/SOC and MBC/SOC)

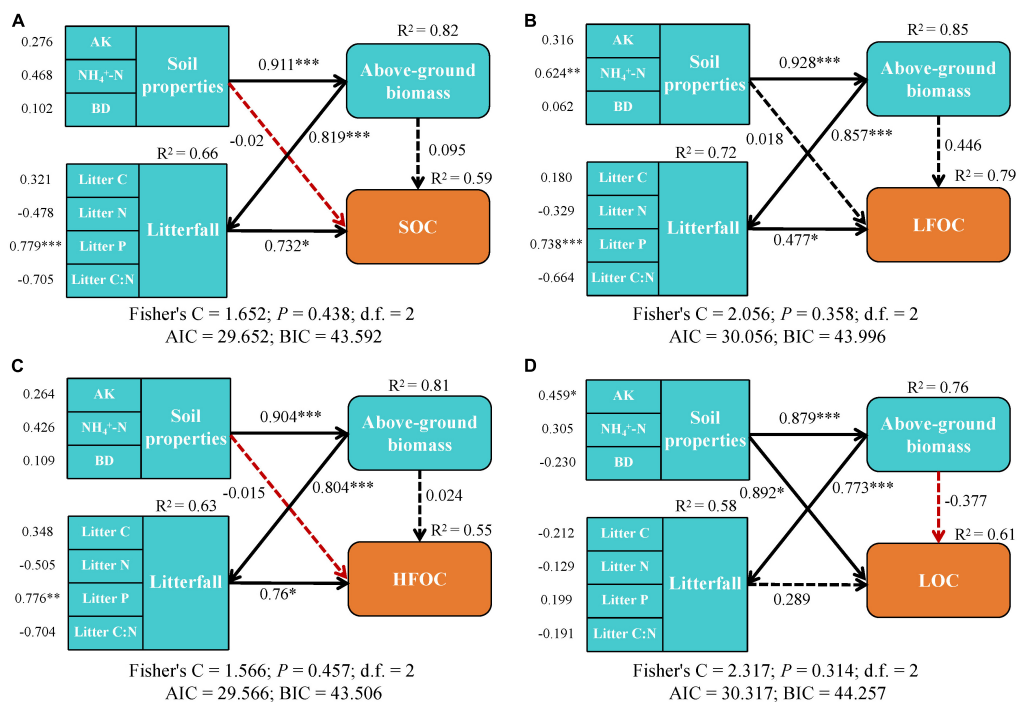


FIGURE 5

PiecewiseSEM pathways of influence the accumulation of the (A) soil organic carbon (SOC), (B) light fraction organic carbon (LFOC), (C) heavy fraction organic carbon (HFOC), and (D) labile organic carbon fractions (LOC). Black line indicates positive correlations, red line indicates negative correlations. The dashed line indicates that the regression results are not significant, and the solid line is significant. Numbers next to arrows represent regression coefficients. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

significantly decreased with increasing soil depth. LOC originates mainly from plant litterfall, soil humus, microorganisms, and plant roots and their secretions. Plant litterfall and roots are mainly distributed in the surface layer of the soil. With increasing depth of the soil layer, soil bulk increases, porosity decreases, and root biomass decreases, leading to a significant decrease in the content of LOC fractions in the lower layers of the soil, indicating that a deeper SOC is better protected and more stable than the surface layer (Berger et al., 2002; Xu et al., 2019). Similarly, a previous study showed that surface soil nutrient changes are more sensitive to disturbances and that deep SOC may be inert (Wattel-Koekoek et al., 2003).

#### 4.4. Relationships between soil physicochemical properties and SOC and its fractions

In this study, a significant positive correlation was observed between SOC and its fractions (LFOC, HFOC, WSOC, ROC, POC, and MBC). This indicates that the content of each SOC fraction depends on the SOC content; thus, a change in the component content can reflect a change in SOC (Li et al., 2014). All SOC and its fractions were significantly positively correlated with TN, TP, and AK, indicating that an increase in soil nutrients favors the accumulation of SOC and its fractions, which is consistent with the results of previous studies (Chen et al., 2018). Some LOC fractions (e.g., WSOC and ROC) were significantly and positively correlated with soil pH, indicating that a decrease in soil pH may reduce the

content of some LOC fractions. Xu et al. (2019) also suggested that pH may affect the turnover rate of some LOC fractions by affecting the microbial species, number, and activity in the soil. However, Zhang et al. (2014) concluded that lowering the pH helps to increase the content of SOC and its active fractions. SOC and its fractions were significantly positively correlated with soil EC, and the effect of EC on SOC and its fractions mainly affected organic carbon fixation by influencing their ability to sorb exchanged ions in the soil. The more the soil organic matter and soil cations, the more negative ions are adsorbed by soil colloids, leading to greater EC (Jing et al., 2006). SOC and its fractions were significantly and negatively correlated with soil BD, indicating that more SOC can be stored in a loose soil environment, which is consistent with the findings of Anh et al. (2014).

In this study, the top three soil environmental factors affecting SOC content and its fractions were AK, NH<sub>4</sub><sup>+</sup>-N, and BD. These three factors accounted for 47.14% of the variance. These findings indicate that these three were the main limiting factors for the accumulation of SOC and its fractions in tropical karst areas. The AK content reflects the K content available to plants in the soil, and NH<sub>4</sub><sup>+</sup>-N is fast-acting N that can be directly absorbed and used by plants (Bao, 2013). During vegetation restoration in tropical karst areas, AK and NH<sub>4</sub><sup>+</sup>-N affect the accumulation of SOC and its fractions by limiting plant growth. A greater BD of the soil results in a more unfavorable accumulation of SOC and its fractions when the downward transport of soil water and organic matter is constrained and the soil permeability becomes poor, resulting in a restricted oxygen supply and reduced activity of soil microorganisms (Zhao Q. et al., 2021). This finding differs from those of previous studies. Pang et al. (2019) suggested that the accumulation of SOC fractions

in karst areas of southwestern China is limited by soil N and P. In contrast, Li et al. (2017) suggested that SOC accumulation is not limited by soil N and that ECa is the main factor affecting soil carbon changes in karst areas. The contents of SOC and its fractions in karst areas are influenced by biotic and abiotic factors that may vary in different areas and vegetation restoration stages. Further investigations are required to determine the factors limiting SOC and its accumulation in karst areas.

#### 4.5. Driving mechanisms of changes in SOC and its fractions

The piecewiseSEM results revealed that Litter P content could directly and significantly affect SOC, LFOC, and HFOC content. The availability of phosphorus can limit plant growth and microbial activity in tropical karst areas, which can limit the accumulation of organic matter in the soil. Plants and microbes can obtain more phosphorus from the litterfall, which stimulates their growth and activity (Staaf and Berg, 1982). This, in turn, can lead to an increased input of organic matter into the soil, resulting in higher levels of SOC, LFOC, and HFOC. Moreover, in karst areas, the soil is often thin and highly weathered with a low capacity to hold nutrients, making it more susceptible to erosion. Litterfall can help stabilize the soil, prevent erosion, and improve soil structure, further enhancing the accumulation of SOC and other organic carbon fractions (Templer et al., 2005).

Here, SOC and HFOC were mainly directly influenced by litterfall, LOC by soil factors, and LFOC by both soil factors and litterfall. Litterfall inputs including fallen leaves, twigs, and other plant materials are major sources of organic matter in tropical karst areas. As litterfall decomposes, organic compounds are released into the soil, contributing to the accumulation of SOC and HFOC. These compounds also contribute to the formation of stable soil aggregates that can enhance SOC and HFOC accumulation. The effectiveness of soil nutrients (AK and  $\text{NH}_4^+\text{-N}$ ) increases microbial activity and the decomposition of organic matter, leading to an increased accumulation of LOC. Soil factors can affect the decomposition and stabilization of LFOC, whereas litterfall inputs can contribute to the accumulation of LFOC.

### 5. Conclusion

This study showed that vegetation restoration significantly increased SOC and its fractions (LFOC, HFOC, WSOC, ROC, POC, and MBC). Meanwhile, SOC and its fractions decreased with increasing soil depth in a tropical karst area in southwest China. Vegetation restoration has great potential to improve the soil environment through the accumulation of SOC and its fractions. These findings indicated that natural vegetation restoration is an important and effective measure for managing and protecting karstic ecological environments. Meanwhile, SOC and its fractions are closely related to soil physicochemical factors. AK,  $\text{NH}_4^+\text{-N}$ , and BD were the main limiting factors in the accumulation of SOC and its fractions. Furthermore, the accumulation of SOC is a dynamic process subject to the combined effects of many factors. Soil properties, above-ground biomass, and litterfall explained most

of the variation in SOC (59%), LFOC (79%), HFOC (81%), and LOC (61%). Soil properties and above-ground biomass significantly affected SOC, LFOC, and HFOC content mainly through indirect effects, while Litter P content could directly and significantly affect SOC, LFOC, and HFOC content.  $\text{NH}_4^+\text{-N}$  and AK of soil factors had direct effects on LFOC and LOC accumulation, respectively. Overall, natural vegetation restoration can significantly improve the carbon sink capacity of tropical karst areas in Southwest China.

### Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

### Author contributions

BZ, GH, and ZZ conceived and designed the study. BZ and CX wrote the first draft of the manuscript. BZ, CX, and CH performed the statistical analysis. BZ, CX, YH, KH, and QP surveyed the sample plots and collected and processed soil and litterfall samples. All authors contributed to the manuscript revision, read, and approved the manuscript.

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### Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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### Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2023.1172062/full#supplementary-material>

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